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Impact of glucocorticoid treatment before pregnancy on glucose homeostasis of offspring exposed to glucocorticoid in adult life

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ABSTRACT

Aims: To explore the impact of GC administration periconceptionally on the glucose metabolism of adult offspring (male and female) and whether this periconception exposure might influence the metabolic outcomes when the offspring are also treated with dexamethasone in adult life.
Materials and methods: Rats received a daily injection of dexamethasone (1 mg/kg, body mass) or saline solution (1 mL/kg body mass) for 7 consecutive days prior became pregnant. Male and female offspring had glucose homeostasis assessed at 3- and 6-month-old and after dexamethasone treatment (1 mg/kg, body mass) or vehicle for 5 consecutive days. Then, murinometric, functional, biochemical, and histomorphometric analyses were performed.
Key findings: Male and female offspring born from rats treated with GC prior to becoming pregnant had none of the murinometric and metabolic outcomes (i.e., body mass, food intake, blood glucose, plasma triacylglycerol, and glucose tolerance) changed up to 6-month-old. None of the expected diabetogenic effects caused by dexamethasone treatment at 6-month of age (i.e., elevation in fasting blood glucose, plasma insulin, triacylglycerol, and albumin, glucose intolerance, insulin insensitivity, augmentation in hepatic glycogen content, and increase in pancreatic islet mass) was observed in offspring born from rats treated with dexamethasone in the pre-pregnancy period. However, periconceptional exposure to GC predisposed the offspring of both sexes to a higher prevalence of augmented fed blood glucose values.
Significance: These results give validity for the use of GC as anti-inflammatory purposes in this critical periconceptional period, but highlight the importance to consider all parental habits when interpreting adult outcomes.

1. Introduction

Due to its anti-inflammatory, antiallergic and immunosuppressive properties, synthetic GCs (i.e., dexamethasone and prednisolone) have received numerous clinical applications [1]. When in excess, GC therapies induce several systemic adverse effects that share many similarities between human and preclinical models, being the diabetogenic effects (i.e., glucose intolerance, peripheral insulin insensitivity, and dyslipidemia) one of the major concerns when considering short- or long-term treatments [2,3]. These adverse effects of GCs may be also exacerbated according to lifestyle (i.e., inactivity and overweight) and can also occur in a sex-specific manner [4].

Based on its diabetogenic actions, GC treatment is prescribed with carefulness. However, there is a group of inflammatory-related diseases where GC is the basis of patients' therapy (i.e., asthma, rheumatoid arthritis, systemic lupus erythematosus) [1]. Moreover, GC may be also part of the immunosuppressive cocktails, which are required for the management of transplanted patients (i.e., renal transplant patients) [1,5]. A portion of these patients may become pregnant and will either require continuation of GC during pregnancy or will have the therapy regimen discontinued [5,6]. In addition, there are also pregnant women under risk of preterm delivery that require GC treatment in the late

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period of gestation [7]. The impacts of antenatal GCs in adult offspring are reasonably known in terms of neurological, cardiovascular and metabolic outcomes [8]. Reduction of birth weight and development of metabolic syndrome-related disorders are between the main outcomes observed in adult life from individuals that were antenatally exposed to GCs [8].

Many of the outcomes observed in the adult life of individuals exposed to prenatal insults (i.e., inactivity, overnutrition) can be also observed by insults occurring before the gestational period (i.e., periconceptional moment). Indeed, several pieces of evidence support the weight of parental influences on offspring health. For instance, periconceptional paternal diet or training behavior may influence the offspring health in adult life [9,10]. This latter evidence emphasizes how epigenetics may contribute to the offspring health either protecting or predisposing them according to parental habits.

The impact of GC given before the gestational period and the possible consequences of that on the offspring is not known. As aforementioned, there is a group of patients under GC therapy that must interrupt them when pregnancy is confirmed [6]. Thus, we aimed to explore the impact of GC administration periconceptionally on the glucose metabolism of adult offspring (male and female). We also aimed to sough the metabolic outcomes of these rats when submitted to GC treatment in adult life to evaluate any possible predisposition acquired through the periconceptional environment. We hypothesized that offspring born from rats exposed to GC periconceptionally might exhibit glucose homeostasis impairments when reaching adult age and that these metabolic impairments could be worsened whether they were exposed to GC. Our main findings revealed that dexamethasone administration just before conception period neither caused any major impact on adult offspring metabolism nor worsened the classical side effects expected when these adult animals were treated with dexamethasone.

2. Materials and Methods

2.1. Ethics statement

The experimental protocol was approved by the Federal University of Santa Catarina Committee for Ethics in Animal Experimentation (PP00782) in accordance with the Brazilian National Council for Animal Experimentation Control (CONCEA) and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Animals

Wistar rats (F0 and F1 groups) were housed in a temperature and humidity-controlled environment and kept on a 12-h light-dark cycle (lights on 06:00 – 18:00 h). All rats had *ad libitum* access to food (commercial standard chow for rats, Nuvilab CR-1; Nuvital, Brazil) and filtered tap water. The animals were supplied by the Federal University of Santa Catarina's Animal Breeding Center.

2.3. Experimental design and groups

Animals (F0) were acclimatized for 8 weeks before being assigned in two groups. F0 rats were composed of 5 females and 2 males for each experimental group (repeated in three different sets of rats), resulting in 30 females and 12 males. Half of the adult females (the MC group) received a daily injection (≈08:00) of saline solution (0.9% NaCl, 1 ml/kg body mass (b.m.), intraperitoneally (i.p.)) and the other half (the MD group) received a daily i.p. injection of dexamethasone (Decadron®, Aché Pharmaceutic Laboratories, Guarulhos, SP, Brazil) (1 mg/kg, b.m.) for 7 consecutive days. At this dose, dexamethasone treatment produces a reduction of body mass, food intake, hyperinsulinemia without insulin resistance and elevation of plasma triacylglycerol in 3-month-old female rats [11]. At the last day of injection, groups of female rats were housed with a male for up to 3 consecutive days (two or three females and one male per cage). Presence of the copulatory plug, confirmed as the presence of spermatozoa in a vaginal lavage, was considered as day 0 of pregnancy and this female rat was transferred to a separate cage. A representative panel containing estrous cycle images and the sperm plug is in Supplemental Fig. 1. Pregnant rats were recomposed to their original groups until parturition. F0 rats had the body mass and glycemia evaluated during the treatment with dexamethasone or saline and during the pregnancy period. The rats that did not become pregnant were either relocated to other projects of the lab with protocols approved by the Federal University of Santa Catarina's Committee for Ethics in Animal Experimentations or euthanized by exposure to CO_2 followed by decapitation. All the dams were restricted to 8 pups until weaning (21 days) (4 males and 4 females with the body masses varying around the median value were considered, and extra animals with the highest and lowest masses were euthanized by direct decapitation). Each group of male and female F1 rats (four or five rats per cage) resulted from a mixture of different dams that means the siblings were not sampled in the same group, being considered the maximum of 2 offspring (male or female) per litter to achieve the final 'n' of animals when necessary [12]. F1 rats were housed up to six months of age in standard conditions as detailed before and had their body mass and food intake determined weekly. Plasma triacylglycerol (blood sampled from the tail of fasted rats) was determined when rats achieved 3- and 6-month-old (before the beginning of GC treatment). An oral glucose challenge was also applied when rats reached either 3-months-old or just before rats had been treated with saline or dexamethasone at about 23-weeks-old as described thereafter. Half of the F1 adult males and females (from MC and MD progenitress) received daily injection (≈08:00) of saline solution (1 ml/kg, b.m., i.p.) for 5 consecutive days, composing the following groups (MCMC; control F1 male from control progenitress), (MCFC; control F1 female from control progenitress), (MDMC; control F1 male from dexamethasone-treated progenitress) and (MDFC; control F1 female from dexamethasone-treated progenitress). The other half of litter received a daily injection of dexamethasone (Decadron®) (1 mg/kg, b.m., i.p.) for 5 consecutive days, composing the following groups (MCMD; dexamethasone-treated F1 male from control progenitress), (MCFD; dexamethasone-treated F1 female from control progenitress), (MDMD; dexamethasone-treated F1 male from dexamethasone-treated progenitress) and (MDFD; dexamethasone-treated F1 female from dexamethasone-treated progenitress). The nomenclature may be also understandable as 'mother control' or 'mother dexamethasone' for every MC or MD appearance in the first 2 characters and 'male offspring control (saline-treated)' or 'male offspring dexamethasone (GC-treated) in the last two characters (MC/MD, respectively). For 'female offspring control' or 'female offspring dexamethasone will be FC and FD in the last two characters, respectively. Body mass was measured daily during dexamethasone or saline treatment. All rats were submitted to an oral glucose tolerance test (oGTT) one day before the euthanasia (in the fifth day of dexamethasone or saline treatment) and to an intraperitoneal insulin challenge in the day of euthanasia (the day after the last dexamethasone or saline administration). The dose and duration of dexamethasone administration were based on previous data where rats (mainly male) developed several alterations in glucose and lipid metabolism (i.e., glucose intolerance, insulin resistance, dyslipidemia) [11,13,14].

2.4. Body mass, food intake, and body mass variation

The body mass was measured daily during the treatment with dexamethasone or saline solution (progenitress and F1 rats) and weekly during the pregnancy period (progenitress) or during housing conditions (F1 rats) in a digital electronic balance (TECNAL, Piracicaba, SP, Brazil). The offspring body mass was also measured in the day of birth.

The food intake was measured weekly after the weaning (21 days) until rats reached six months of age. The determination of food intake was done by weighing the remaining chow that was discounted from the total of chow available 24 h before and the mass difference represents the daily amount ingested per cage [15]. The average amount of food ingested per animal was obtained by the following formula [(total chow (g) ingested in the cage/number of rats per cage)/individual rat mass (g)]*100. The results were expressed as grams (g) of food ingested per 100 g of body mass. Body mass variation (% change in body mass) was determined according to the formula [(final value - initial value)/initial value]*100.

2.5. Oral glucose challenge and oral glucose tolerance test (oGTT)

The oral glucose challenge was applied in the third and sixth month of age in fasted (≈12:00 h) F1 male and female rats (at this point rats were grouped only by sex and progenitors' treatment – MC or MD). Rats had the tip of the tail cut (no more than 1 mm) for blood collection at about 08:00. The first blood drop was discarded, and the second blood drop was used for the determination of basal blood glucose using a glucometer (Accu-Chek Performa; Roche Diagnostics, GmbH, Mannheim, Germany). Immediately, 50% glucose solution pre-warmed at 36 °C (2 g/kg, b.m.) was administered (oral gavage, intragastric) and blood samples were then collected from the tail of the rat at min 60. The oGTT was performed in the day before euthanasia (in the fifth day of dexamethasone or saline administration) in fasted (≈12:00 h) F1 male (MCMC, MCMD, MDMC, and MDMD) and female groups of rats (MCFC, MCFD, MDFC, and MDFD). Determination of basal glycemia and glucose administration was done as for the oral glucose challenge. Blood glucose values were next taken on minutes 30, 60 and 120 as previously described [11,15]. Area-under-glucose-curve (AUC) was obtained after normalization by the initial blood glucose values. The data analyses were also interpreted based on initial baseline values; for male rats, they were divided into two groups based on basal glycemia, being one group with basal glycemia higher than 200 mg/dL and another group with basal glycemia lower than 200 mg/dL. Female rats were also divided into two groups, being one group with basal glycemia higher than 150 mg/dL and another group with basal glycemia lower than 150 mg/dL.

2.6. Intraperitoneal insulin challenge

The i.p. insulin challenge was performed in the day of euthanasia in fed rats at about 08:00. Blood collection at min 0 occurred as for oGTT. Then, animals received an i.p. injection of recombinant human insulin pre-warmed at 36 °C (Biohulin® 2 IU/kg b.m.). After 25 min, blood glucose value was determined from the tail tip as for min 0. The data analyses were also interpreted based on initial baseline values; for male rats, they were divided into two groups based on basal glycemia, being one group with basal glycemia higher than 250 mg/dL and another group with basal glycemia lower than 250 mg/dL. Female rats were also divided into two groups, being one group with basal glycemia higher than 200 mg/dL and another group with basal glycemia lower than 200 mg/dL.

2.7. Euthanasia, organ masses and biochemical analyses

The euthanasia occurred 6 h after the i.p. insulin challenge and was done by exposure to CO_2, followed by decapitation. Organs of interest (adrenals, liver, pancreas, spleen, and visceral fat depots) were carefully removed and weighed on an electronic analytical balance. Fragments of pancreas and liver were used for histological procedures as described in detail thereafter. The mass of the organs was normalized by the total body mass. The blood from the trunk was collected in a glass tube containing EDTA-NaF (Glistab, Labtest, Lagoa Santa, MG, Brazil) and then centrifuged at 1,500 rpm for 10 min at room temperature (Eppendorf 5810R). Aliquots of plasma stored at −20 °C for further quantification of insulin by the AlphaLisa® system (PerkinElmer, Waltham, MA, USA, cat. Nº AL204), triacylglycerol, albumin, TGO and TGP (commercial kits from Biotécnica, Varginha, MG, Brazil) according to manufacturer's instructions and previous publications [11,15]. Blood sample (40 µl) for determination of plasma triacylglycerol at 3-month-old was obtained through the tip of the tail and processed in a glass tube containing EDTA-NaF (Glistab) as described before.

2.8. Markers of liver oxidative stress and hepatic glycogen and triacylglycerol contents

Lipid hydroperoxidation (LOOH), protein carbonylation, catalase, and superoxide dismutase activity were quantified in liver fragments according to previous publications [16–19] with minor modifications. The hepatic glycogen and triacylglycerol content were done according to previous publications [14,15,20].

2.9. Hepatic morphology and islet mass

For hepatic morphology, liver fragments (from the same lobe in all animals) were collected and fixed in 10% buffered formalin, pH 7.4, for 24 h, dehydrated and embedded in paraffin. Representative tissue sections (5 µm) were obtained on a rotating microtome (Leica, IL, USA) and placed on glass slides. After, the slides were submitted to the staining procedure of Hematoxylin and Eosin (HE) for posterior morphological evaluation including verification of the presence of lipids and glycogen according to previous publications [15,20]. Representative images were taken in Opticam 0400S microscope (OPTICAN INC.; São Paulo, SP, Brazil), with 40× and 400× of magnification. For islet mass determination, pancreas fragments (splenic region) were collected, fixed and embedded as for liver fragments. Representative tissue sections (5 µm) were obtained on a rotating microtome (Leica) and placed on glass slides. Subsequently, the slides were submitted to the staining procedure of HE and posteriorly scanned in the AxioScan automatic slide scanner (ZEISS, Oberkochen, Germany) for subsequent morphometric analyses. The total area of the pancreas and pancreatic islets were calculated using the ZEN software (ZEISS) and the sum of the areas corresponding to the pancreatic islets was divided by the total pancreas area and multiplied by 100 to obtain the percentage of the endocrine pancreas in the section. To obtain the absolute islet mass, the entire pancreas mass was multiplied by the percentage of islet (endocrine pancreas) in the section and expressed as mg. Representative images were taken in OLYMPUS IX83 microscope (OLYMPUS; Tokyo, Japan) with 40× and 200× of magnification.

2.10. Statistical analysis

All analyses were performed using GraphPad Prism Version 6.01 software (GraphPad Inc., La Jolla, CA, United States). The results were expressed as the mean ± SD for parametric data or median and interquartile ranges for non-parametric data of the number (n) of animals. The symmetry of the data was tested by Kolmogorov–Smirnov, Shapiro–Wilk, and D'Agostino and Pearson omnibus normality tests. It was considered symmetric if approved by at least one of three tests. Analysis of variance (2-way ANOVA) followed by Tukey's *post hoc* test was used for multiple comparisons. When indicated in the figure legend, paired or unpaired Student's *t*-test (with Welch correction when necessary) and paired Mann-Whitney or unpaired Wilcoxon test were applied for some parametric and non-parametric data, respectively. Fisher's exact test was also applied when indicated in the figure legend for contingency analysis. Extreme studentized deviate method was applied to determine whether any value had reached significant outlier (Grubb's test, available online on GraphPad QuickCalcs). Significance was set at $p < 0.05$.

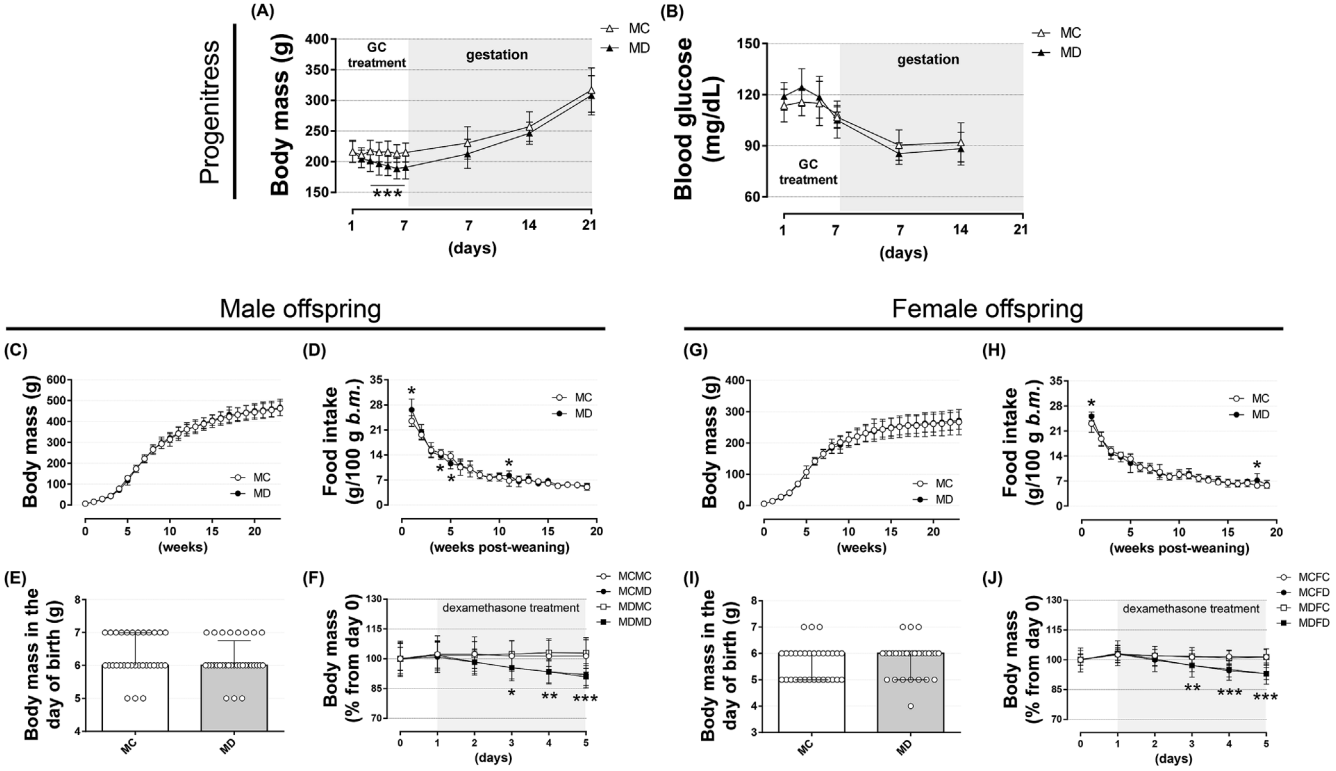

Fig. 1. *Impact of prepregnancy dexamethasone treatment in the pregnant and offspring parameters.* Body mass **(A)** and blood glucose values **(B)** during and after daily i.p. dexamethasone administration (1 mg/kg, *b.m.*, for 7 consecutive days). In **(C–E)** body mass and food intake observed weekly and body mass in the day of birth in male offspring. In **(G–I)** body mass and food intake observed weekly and body mass in the day of birth in female offspring. Body mass during dexamethasone administration in both male **(F)** and female **(J)** rats. Results are expressed as mean ± SD **(A-D, F–H,** and **J)** and as median ± interquartile range (asymmetric/nonparametric values) **(E** and **I)**. The asterisk indicates a significant difference compared to the respective control groups using unpaired Student's *t*-test (with Welch's correction if necessary) in **A-D, G,** and **H**, unpaired Man-Whitney in **E** and **I** and ordinary two-way ANOVA with Tukey's *post hoc* in **F** and **J**. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

3. Results

3.1. Dexamethasone exposure before pregnancy period had no impact on the pregnant body mass and blood glucose, and on the offspring body mass at birth

Dexamethasone administration in the prepregnancy period resulted in a loss of body mass in the last three days of treatment ($p < 0.05$), but this was not enough to impair the body mass gain during the pregnancy period (Fig. 1A). Dexamethasone treatment neither altered fed blood glucose during the GC treatment nor influenced the glycemia during the entire pregnancy period (Fig. 1B). The number of total newborns and the proportion of male and female was unaltered by the periconceptional treatment with dexamethasone. Both saline- and dexamethasone-treated rats had an average number of 5 ± 1 male and 5 ± 1 female pups. Male and female offspring from progenitress treated with dexamethasone exhibited similar body mass at birth in relation to their controls (Fig. 1E,I, respectively) and developed normal body mass gain and food intake up to 23 weeks of age (when dexamethasone treatment began) (Fig. 1C,D,G,H respectively). Body mass variation values from the birth to the 23rd week of age were 7,264 ± 799% and 7,594 ± 956% for male offspring, MC and MD groups, respectively, and 4,498 ± 481% and 4,579 ± 655% for female offspring, MC and MD groups, respectively. Dexamethasone treatment in F1 male and female rats at 6-month-old led to lower body mass compared to their respective controls (Fig. 1F,J) ($p < 0.05$) and the progenitress exposure to the GC had no additional impact on this parameter.

3.2. Dexamethasone administration in the periconceptional period caused no influence in the basal and post-glucose load glycemic values in F1 male and female adults at 3- or 6-month-old

To evaluate whether prepregnancy exposure to GC could affect glucose homeostasis in adult life, we performed a glucose challenge in the male and female offspring. Fasting glycemia measured in the third (Fig. 2A,B,E,F) and in the sixth (Fig. 2C,D,G,H) month of age was unaltered in male and female rats born from progenitress exposed to dexamethasone periconceptionally (MD groups), compared to their respective controls (MC groups). Blood glucose evaluated after 60 min of an oral *bolus* of glucose was not altered in both male and female offspring at 3- or 6-month-old indicating no influence of GC whether it was given just before the pregnancy period (MD groups). The plasma triacylglycerol was not affected in any group at 3- or 6-month-old (data not shown).

3.3. Prepregnancy dexamethasone exposure had a minor impact on metabolic offspring outcomes when they receive GC treatment in adult life

Having observed that glucose homeostasis was unaltered in offspring born from progenitress exposed to GC periconceptionally, we next treated F1 male and female rats with dexamethasone at 6-months of age to investigate whether there was any vulnerability that could exacerbate the well-known metabolic side effects caused by GC excess when administered in adult rats. Results from oGTT revealed no additional impact of GC exposure before pregnancy in the glucose intolerance observed in the adult male offspring treated with dexamethasone (Fig. 3A,B), but had a minor influence in the glucose tolerance in the adult female offspring treated with dexamethasone (Fig. 3G,H)

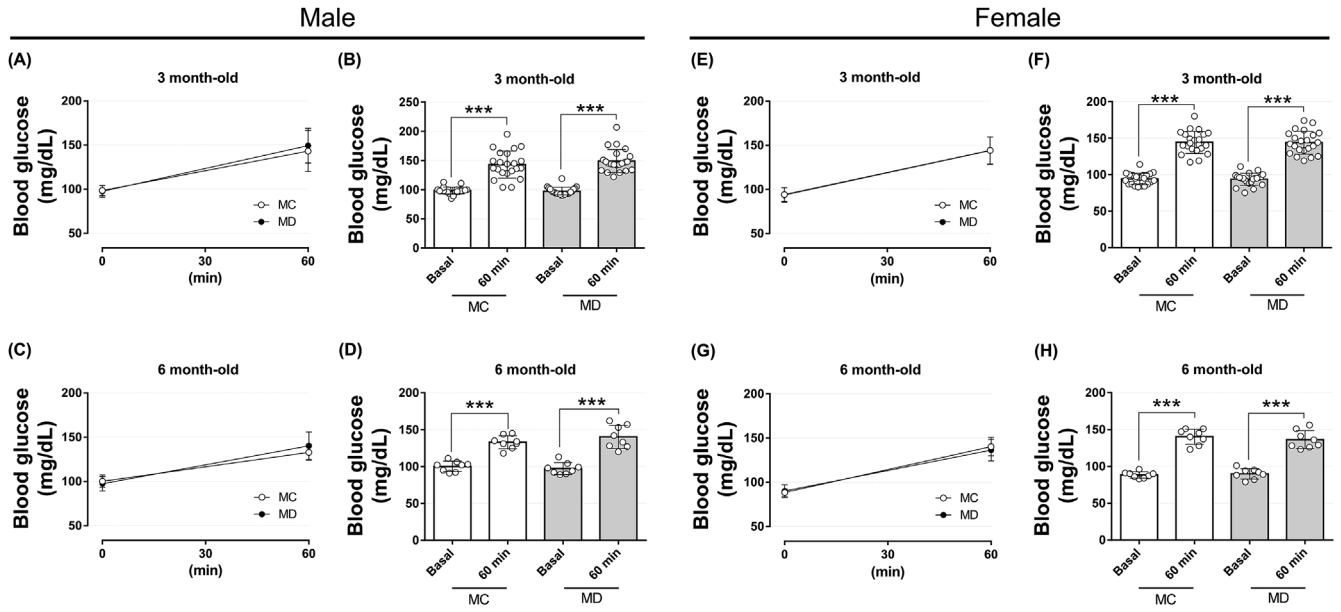

Fig. 2. *Basal blood glucose and response to oral glucose challenge in the offspring at 3- and* 6-month-*old.* Basal and post oral glucose load (2 g/kg, *b.m.*) glycemia in male **(A,B)** and in female **(E,F)** offspring with 3-months-old. Basal and post oral glucose load (2 g/kg, *b.m.*) glycemia in male **(C,D)** and in female **(G,H)** offspring with 6-months-old. Results are expressed as mean ± SD. The asterisk indicates a significant difference compared to basal values using paired Student's *t*-test. ***$p < 0.001$.

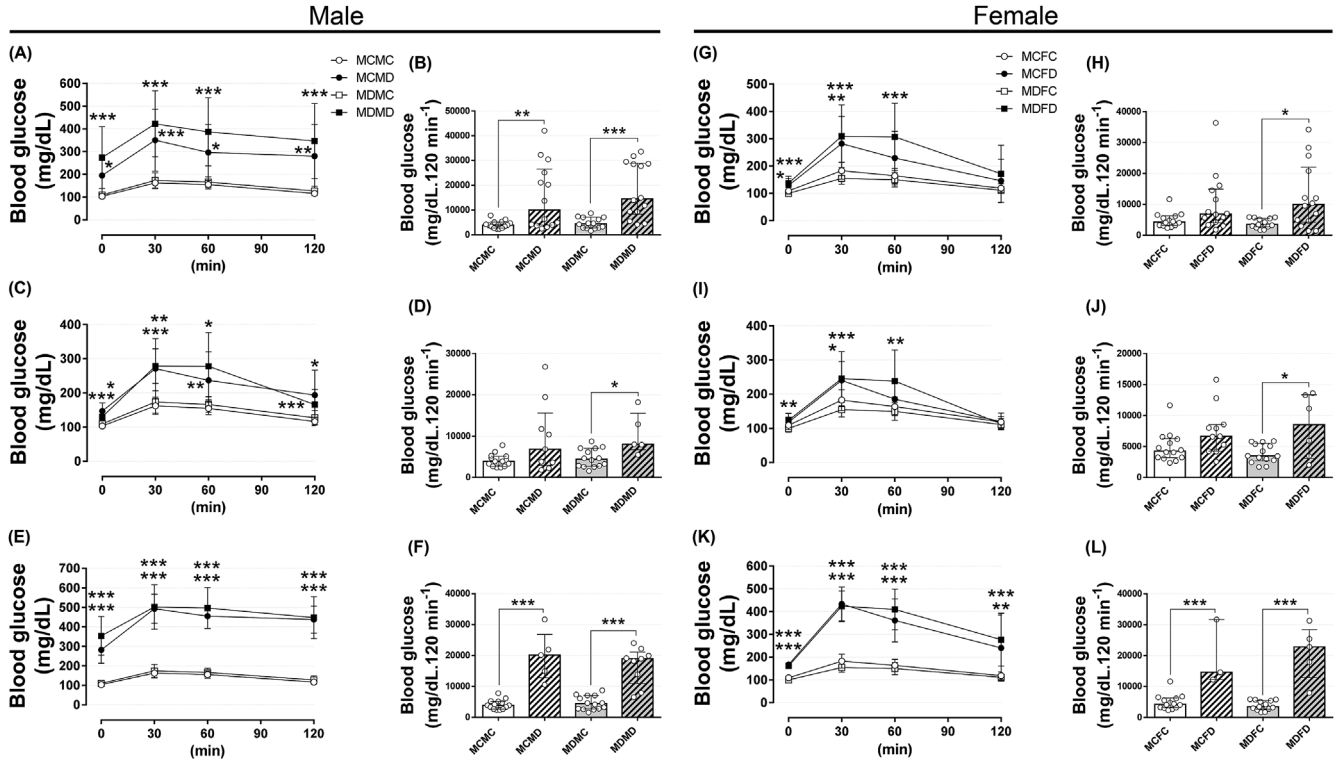

Fig. 3. *Glucose tolerance in the offspring after dexamethasone treatment in adult life.* The oral glucose tolerance test (2 g/kg b m.) performed in the fifth day of dexamethasone (1 mg/kg, *b.m.*, *i.p.*) or saline (0.9% NaCl) administration in the adult male **(A)** and female **(G)** offspring rats. Blood glucose values in 'A' were stratified according to basal glycemia lower **(C)** or higher **(E)** than 200 mg/dL in male rats and the respective area-under-glucose-curve (AUC) **(B,D,F)**. Blood glucose values in 'G' were stratified according to basal glycemia lower **(I)** or higher **(K)** than 150 mg/dL in female rats and the respective AUC **(H,J,L)**. Results are expressed as mean ± SD in **A,C,E,G,I,** and **K** and as median ± interquartile range (asymmetric/nonparametric values) in **B,D,F,H,J,** and **L**. The asterisk indicates a significant difference compared to the respective control groups (effect of dexamethasone treatment in adult life) using ordinary two-way ANOVA with Tukey's *post hoc*. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Observation: time-points with overlapping asterisks mean difference of both groups treated with dexamethasone in the adult period (*i.e.*, MCMD and MDMD) *vs.* their respective control groups (MCMC and MDMC), respectively. The same for the female group. Please, see Material and Methods for details of experimental design and description of the groups.

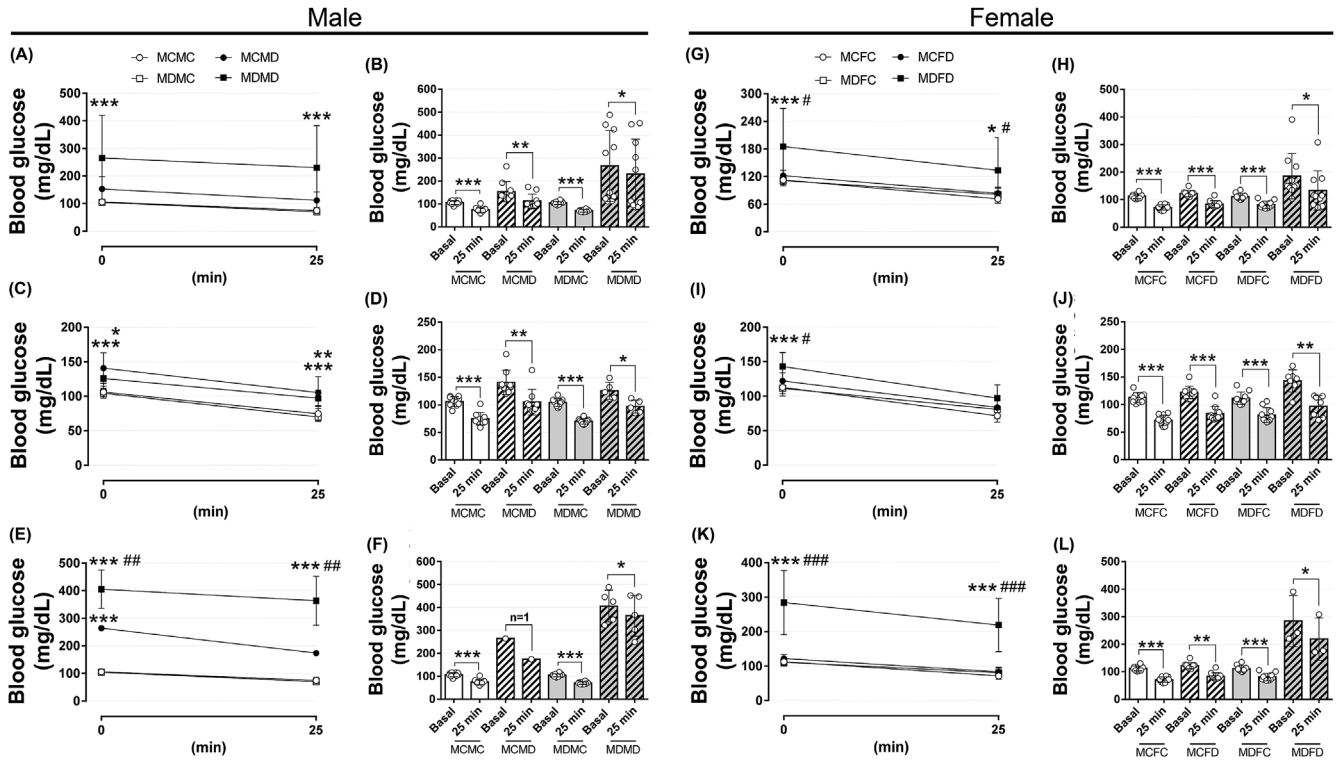

Fig. 4. *Insulin responsiveness in the offspring after dexamethasone treatment in adult life.* The intraperitoneal insulin challenge (2 IU/kg *b.m.*) performed after five days dexamethasone (1 mg/kg, *b.m.*, *i.p.*) treatment (day of euthanasia) in the adult male **(A)** and female **(G)** offspring rats. Blood glucose values in 'A' were stratified according to basal glycemia lower **(C)** or higher **(E)** than 250 mg/dL in male rats and the respective post insulin load comparisons **(B,D,F)**. Blood glucose values in 'G' were stratified according to basal glycemia lower **(I)** or higher **(K)** than 200 mg/dL in female rats and the respective post insulin load comparisons **(H,J,L)**. Results are expressed as mean ± SD. Asterisks indicate a significant difference compared to the respective control groups (effect of dexamethasone treatment in adult life) and number signs indicate a significant difference compared to the respective control groups (effect of dexamethasone treatment in pre-pregnancy) using ordinary two-way ANOVA with Tukey's *post hoc* in **A,C,E,G,I,** and **K** or paired Student's *t*-test or paired Wilcoxon test for **B,D,F,H,J,** and **L**. $^{*,\#}p < 0.05$, $^{**,\#\#}p < 0.01$ and $^{***,\#\#\#}p < 0.001$. Observation: time-points with overlapping asterisks mean difference of both groups treated with dexamethasone in the adult period (*i.e.*, MCMD and MDMD, Fig. 4C) *vs.* their respective control groups (MCMC and MDMC), respectively. Please, see Material and Methods for details of experimental design and description of the groups.

($p < 0.05$). Stratification of these data by basal glycemia (higher or lower than 200 mg/dL for male; Fig. 3C–F) and (higher or lower than 150 mg/dL for female; Fig. 3I-L) revealed a minor negative impact of periconceptional GC exposure to glucose tolerance in both male and female offspring having lower baseline glycemic values (Fig. 3C,D for male and I,J for female offspring) ($p < 0.05$).

Blood glucose decay in response to exogenous insulin *bolus* was not altered in F1 male and female offspring born from dexamethasone-treated progenitress. Both male and female offspring treated with dexamethasone in adult life, independent of periconceptional context, exhibited similar insulin responsiveness after an i.p. insulin load (Fig. 4A, B for male and G,H for female rats). As for oGTT, insulin challenge data were also stratified (see methods for details) and it was possible to observe glucose decay after insulin administration in any context evaluated (Fig. 4C–F for male and I-L for female, respectively). However, either male or female fed blood glucose levels were higher in MDMD and MDFD *vs.* MCMD and MCFD, respectively, that were pronounced in those rats that had higher basal blood glucose values.

The contingency analysis revealed a higher frequency of rats with higher blood glucose levels whether they were treated with GC in adult life and was born from GC-treated progenitress. Fig. 5A,C were extracted from the oGTT experiments for male and female, respectively; and Fig. 5B,D were extracted from the insulin challenge experiments for male and female, respectively.

3.4. GC excess in the prepregnancy period had no major impact on overall biochemical parameters, hepatic oxidative stress markers and metabolic organs' masses that were altered by dexamethasone treatment in adult offspring

As expected, dexamethasone treatment in adult life resulted in higher plasma insulin values in both male and female offspring ($p < 0.01$), being quite pronounced in MDFD group of female rats (Suppl. Fig. 2A, D for male and female offspring, respectively). Plasma triacylglycerol and albumin values were higher in male and female rats treated with dexamethasone in adult life, with no additional impact caused by progenitress exposure to GC before pregnancy period (Suppl. Fig. 2B,C and E,F for male and female offspring, respectively). Markers of oxidative stress in liver (i.e., reduced catalase activity, increased lipid hydroperoxidation and plasmatic TGP) were affected by GC treatment in male and female adult offspring with no additional influence of GC in prepregnancy period (Suppl. Fig. 3A-L), except for a pronounced plasma TGP elevation in female offspring. Values of relative organ masses were also similar between male and female offspring treated with dexamethasone in adult life, being adrenal and spleen hypotrophy and liver hypertrophy the main changes observed with no major influence of GC in prepregnancy period (Suppl. Fig. 4A–H).

3.5. Dexamethasone excess in the periconceptional period did not worsen liver histology and substrate contents or islet mass alterations that were caused by GC treatment in adult offspring

The negative impact of GC treatment in adult life in the hepatic

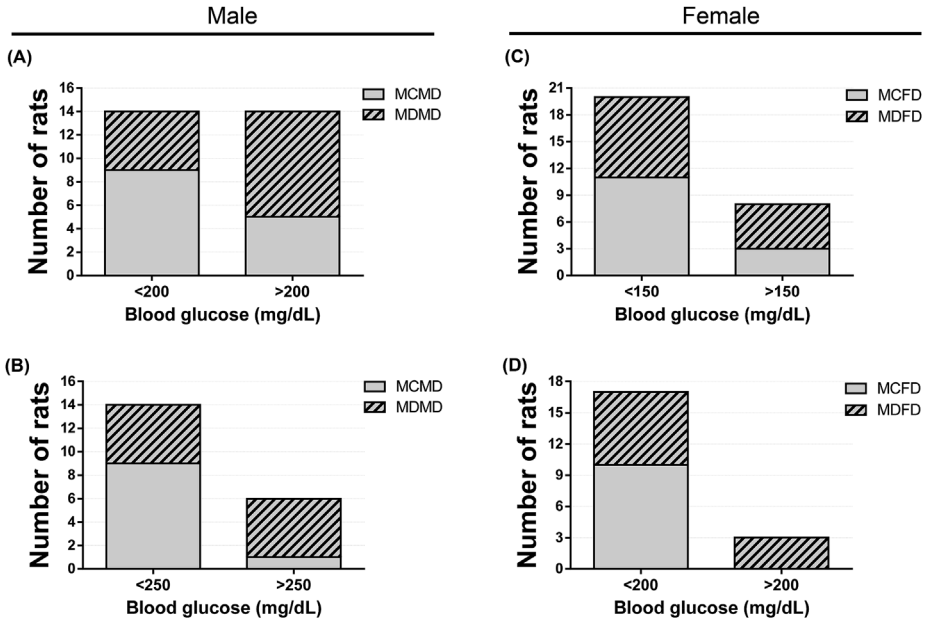

Fig. 5. *Profile of fasting and fed blood glucose in the offspring after dexamethasone treatment in adult life.* The number of male rats having fasting blood glucose lower or higher than 200 mg/dL **(A)** and female rats having fasting blood glucose lower or higher than 150 mg/dL **(C)** (data obtained from baseline blood glucose values during oGTT experiment). The number of male rats having fed blood glucose lower or higher than 250 mg/dL **(B)** and female rats having blood glucose lower or higher than 200 mg/dL **(D)** (data obtained from baseline blood glucose values during insulin challenge experiment). Fisher exact test was applied. Please, see Material and Methods for details of experimental design and description of the groups.

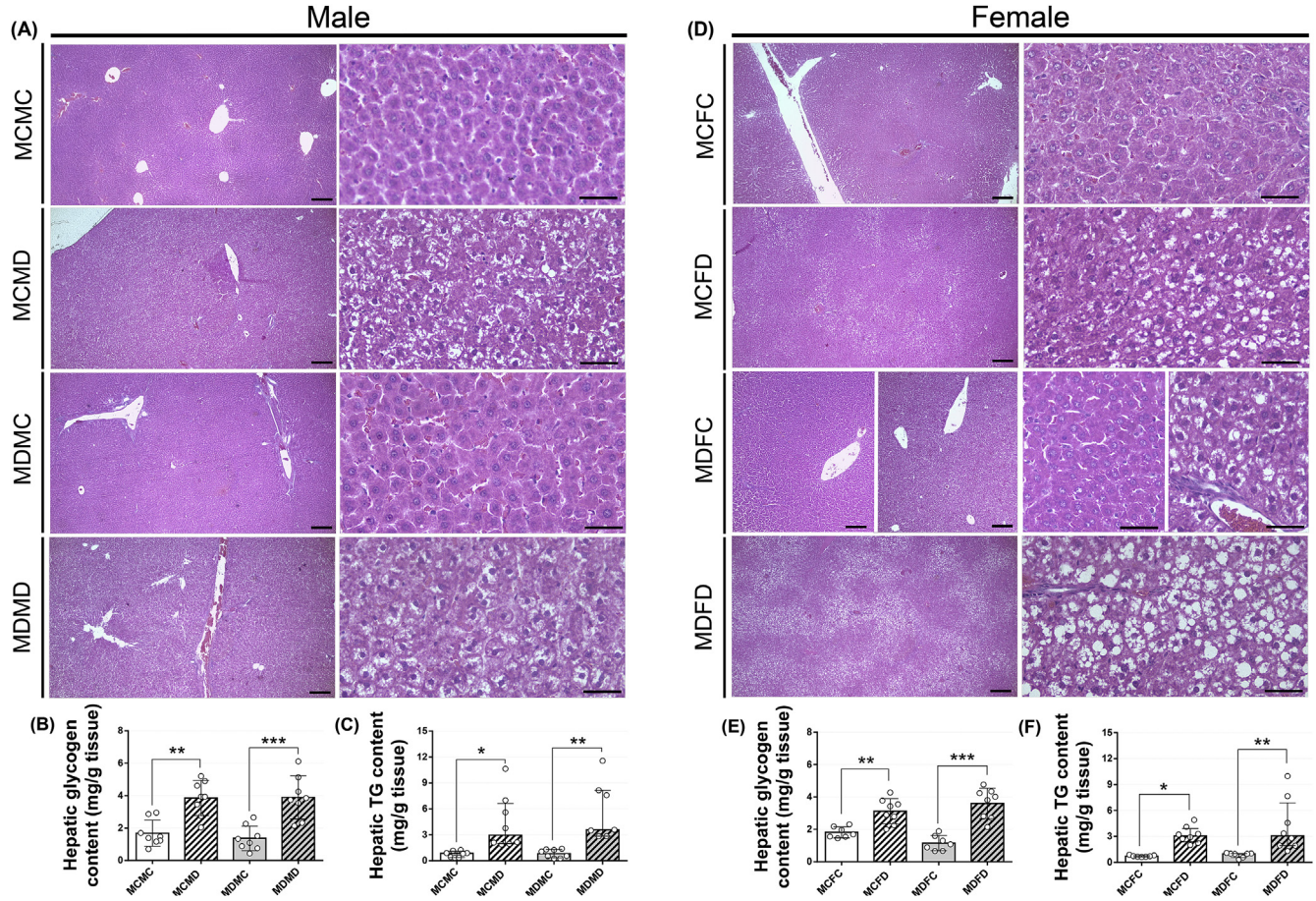

Fig. 6. *Hepatic glycogen and triacylglycerol contents in the offspring after dexamethasone treatment in adult life.* Representative images of the liver section with final magnitudes of 40× and 400× in the male **(A)** and **(D)** female groups. Some rats from the MDFC group exhibited signs of glycogen deposition. Sections were stained with Hematoxylin & Eosin. In (**B** and **E**) the glycogen and in (**C** and **F**) the triacylglycerol content in the liver quantified from liver fragments of the male and female groups, respectively. Results are expressed as mean ± SD in **B** and **E** and as median ± interquartile range (asymmetric/nonparametric values) in **C** and **F**. The asterisk indicates a significant difference compared to the respective control groups (effect of dexamethasone treatment in adult life) using ordinary two-way ANOVA with Tukey's *post hoc*. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Please, see Material and Methods for details of experimental design and description of the groups. **TG;** triacylglycerol.

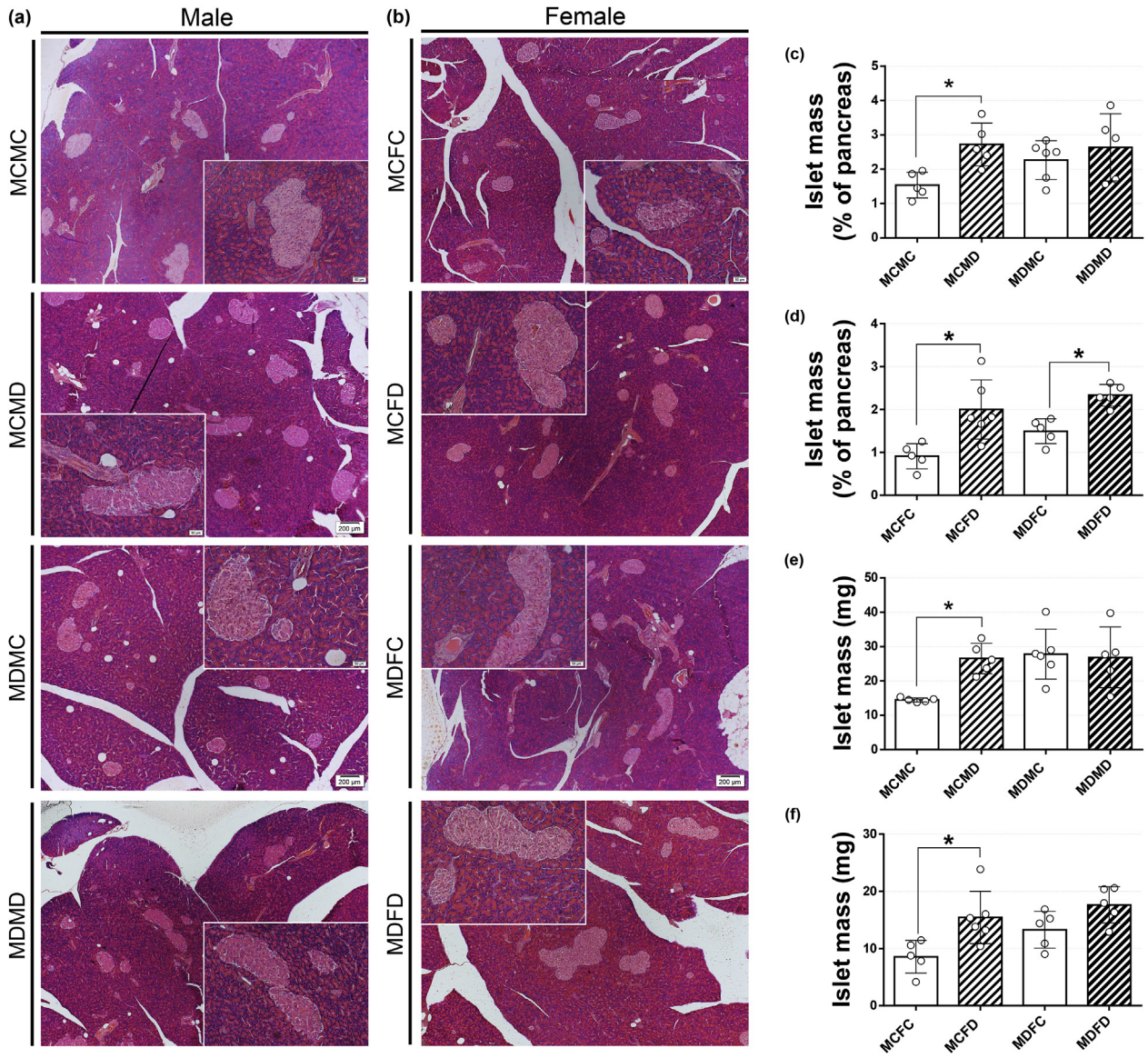

Fig. 7. *Pancreatic islet mass in the offspring after dexamethasone treatment in adult life.* Representative images of pancreas section with final magnitudes of 40× and 200× (inserts) in the male **(A)** and **(C)** female groups. Sections were stained with Hematoxylin & Eosin. In (**B** and **D**) the relative islet mass (%) in the male and female groups, respectively. In (**E** and **F**) the absolute islet mass (mg) in the male and female groups, respectively. Results are expressed in mean ± SD. The asterisk indicates a significant difference compared to the respective control groups (effect of dexamethasone treatment in adult life) using ordinary two-way ANOVA with Tukey's *post hoc*. *$p < 0.05$. Please, see Material and Methods for details of experimental design and description of the groups.

oxidative stress markers was associated with the presence of glycogen and triacylglycerol content in the liver in both male and female offspring (Fig. 6A-F). Adult female treated with saline and born from GC-treated progenitress exhibited glycogen accumulation in the liver (in 3 of 6 rats evaluated) (Fig. 6D, see MDFC group).

In the male offspring treated with dexamethasone, independent of progenitress influence, islet mass was higher as expected by the effect of GC excess (Fig. 7A,C,E). The MDMC group of rats had islet mass values equivalent to that of MDMD (Fig. 7E). In the female offspring, GC treatment in adult life resulted in higher islet mass in both MCFD and MDFD groups (Fig. 7B,D,F) and, as for male rats, prepregnancy GC exposure influenced islet mass of the MDFC (Fig. 7F).

4. Discussion

GC adverse effects are concerns that reflect on therapy regimen since either excess or prolonged treatments are commonly associated with diabetogenic actions [1,2]. The demonstration that antenatal exposure to GC excess causes metabolic programming and may predispose offspring to future diseases has stimulated the investigation of derivative experimental designs in an attempt to test whether they can predict harmful consequences in adult life of the offspring. In our study, we exposed rats to dexamethasone treatment in the periconceptional window and demonstrated that male and female offspring exhibit minor dysfunctions in their glucose and lipid metabolism. Overall, they had neither modification of their birth weight, body mass variation, food intake, plasma triacylglycerol, blood glucose and glucose tolerance at 3- and 6-months-old nor any relevant exacerbation of those diabetogenic actions of GC when exposed to it at 6-month-old. The minor aspect found was the higher prevalence of offspring (male and female) on high glycemic levels after GC treatment at 6-month-old.

The reduction in the body mass and unaltered random blood glucose in the young female rats treated with dexamethasone just before the pregnancy is in accordance with the expected outcomes already fully demonstrated in other studies [11,21]. This is because of several aspects, being the main of them the anorexigenic effect of insulin and

leptin on hypothalamus with a consequent reduction in the food intake since these rats develop higher levels of plasma insulin and leptin [14,15,21]. The normal blood glucose is explained by augmented beta-cell function and mass that counteract any eventual reduction in the peripheral insulin sensitivity [11,14,15].

Numerous studies demonstrated that prenatal exposure to high GC levels led to lower body mass at birth, which use to be normalized during the development of these animals [12,22,23]. Here, we demonstrated that dexamethasone treatment in the prepregnancy period neither influence the body mass of the pups at birth nor the body mass variation of the offspring up to 6-month-old. This is probably explained by the fact that even a residual amount of dexamethasone in pregnant rats was not enough to affect fetus development. There is evidence that restriction in body weight did not occur in rats whether the GC is elevated either in the first or in the second period of gestation [24]. This latter study highlighted that the third period of gestation seems to be the most susceptible window that rebounds in adult offspring outcomes. Our glucose homeostasis data corroborated this evidence as both blood glucose and glucose tolerance were unaffected either in male or female offspring at 3- and 6-month old. Despite that, we do not discard the possibility that for other biological parameters this periconceptional exposure to GCs results in any impact. Nonetheless, we based our model in short-term exposure and, perhaps, in longer therapies the impact of them might be different.

Since none of the classical diabetogenic effects was observed in our offspring up to 6-month-old, we challenged them with a regimen of dexamethasone that is well-known to generate a prediabetes-like context (i.e., glucose intolerance and dyslipidemia) expecting to reveal any metabolic vulnerability. As can be observed in our data with glucose and insulin load, again, these rats exhibited minimal impairments on those side effects expected by the GC treatment. However, the higher fed blood glucose levels and the higher prevalence of rats with augmented blood glucose levels brought our attention up. This is a point that merit attention and perhaps might explain why certain patients are more prone to certain collateral effects of medications. In line with this, our data stratification revealed two patterns of GC response during glucose or insulin load. This dual pattern of response to dexamethasone was already demonstrated in rats in a study by Ogawa and colleagues [25] many years ago and reiterates the inter-individual variabilities that can be affected by many parameters, including the parental epigenetic influences. Higher fed blood glucose values in offspring born from GC-treated progenitress is possibly a result of a less competent beta-cell function and/or mass compensation. Although both offspring had upregulated their islet-mass - that is constituted of about 75% of beta cells in rats [26] – this was in the same level of beta-cell mass upregulation observed in those rats not exposed to GC in adult life (MDMC and MDFC groups). This is a point that indicates any limitation in the compensatory increase of beta-cell mass caused by GCs. Importantly, other parameters that could give us additional information and are known to be impacted by GC treatment such as glucagonemia [27], insulin secretion [11,14], and insulin clearance [28] were not assessed in this study. However, it is still important to emphasize that the impact of periconceptional exposure to GC was minimal on overall glucose homeostasis.

Another known set of metabolic parameters that are affected by GC treatment includes plasma triacylglycerol and liver histology [14,15,20]. Prepregnancy administration of dexamethasone neither affect the offspring plasma triacylglycerol values and liver function nor worsened the alterations in lipid metabolism and liver function caused by dexamethasone (i.e., elevation in plasma triacylglycerol values, accumulation of glycogen and triacylglycerol in the liver, reduction in catalase activity and increase in lipid hydroperoxidation as well). These data reiterate the above-mentioned explanation that at this periconceptional exposure GCs seem to not have enough strength to generate any major health concerns in adult life.

Of note, by exploring 'whether' but not 'how' periconceptional GC exposure might affect the metabolism of adult offspring, we prioritize more an overall functional analysis than cellular and molecular aspects in detail that might bring some information not captured here.

5. Conclusion

In summary, dexamethasone administration just before conception period neither caused any major impact on adult offspring metabolism nor worsened the diabetogenic action of GC expected when these adult rats were treated with dexamethasone. Thus, we conclude that periconceptional exposure to GC has a minor impact on male and female offspring glucose homeostasis in adult life, except for an increase in the number of animals with high fed blood glucose value. These results give validity for the use of GC as anti-inflammatory purposes in this critical periconceptional period, but highlight the importance to consider all parental habits when interpreting adult outcomes.

Data availability

The datasets generated to support the findings of this study are available from the corresponding author upon reasonable request.

Authorship contributions

Conceptualization: A.R.; Data curation: A.R; Formal analysis, A.R.; Funding acquisition: A.R.; Investigation: F.N.S., H.S.B., M.A.B., and F.A.G.; Methodology: A.R., F.N.S., H.S.B., M.A.B., and F.A.G.; Project administration: A.R.; Resources: A.R.; Software: A.R.; Supervision: A.R.; Validation: A.R.; Visualization: A.R.; Roles/Writing - original draft: A.R.; Writing - review & editing: A.R., F.N.S., H.S.B., M.A.B., and F.A.G.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116913.

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